

Reproductive Biology of a New Hesionid Polychaete From the Great Barrier Reef

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Abstract. We describe *Lizardia hirschi*, a new hesionid genus and species, from shallow water on the Great Barrier Reef. It is characterized by small size (maximally around 2 mm long) and by males with paired penes on the last segment or the pygidium. The sperm are elongated, with a conical acrosome; extended, cylindrical nucleus; and three mitochondria. The females have three to four pairs of eggs in segments 10–13, up to 150 μm in diameter. The female reproductive system consists of spermathecae, situated in the notopodia of segments 10–12, and oviducts opening ventrally on segment 11. Fertilization may be internal. The female (but not the male) reproductive system appears to be homologous to that in another small hesionid, *capricornia*. The phylogenetic position of *L. hirschi* within Hesionidae is currently uncertain due to the retention of many apparently larval features in the adults.

Introduction

Hesionids are generally not provided with external genital organs (Pleijel, 1998, 2001). Recently, however, two new hesionids were described (Westheide *et al.*, 1994, Pleijel and Rouse, 2000), both of which have external genital organs. In *Sinohesione genitaliphora* Westheide, Purschke and Mangerich, 1994, the males have a pair of penes situated on the ventral side of the neuropodia of segment 14, and the females have paired openings of the spermathecae

in the same position but on segment 16. In *capricornia* Pleijel and Rouse, 2000 (see this description for spelling of taxon name with the initial letter in lower case), the males have a pair of large penes situated at the bases of the neuropodia of segment 9, and the females have spermathecae in the notopodia of segments 11 and 12. Based on the phylogenetic relationships (Pleijel and Rouse, 2000), the external reproductive organs in these two taxa were considered nonhomologous. Here we introduce a third taxon with external genital organs, occurring as paired penes on the posterior end on the males, collected from several localities in shallow water on the Great Barrier Reef. Similar to *capricornia*, this new hesionid is very small, reaching only 2 mm in length, and has a number of characters in common with juvenile stages of other hesionids.

Materials and Methods

Specimens were extracted from scuba-collected sand and gravel samples by decantation through a 250- μm sieve and relaxed in a mixture of 7% (by weight) of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (in distilled water) and filtered seawater (see Rouse and Pleijel, 2001, for details). Relaxed specimens were studied alive, then processed for long-term storage and LM (light microscopy), SEM (scanning electron microscopy), or histology and TEM (transmission electron microscopy). For long-term storage, specimens were fixed in 10% formaldehyde (*i.e.*, 25% formalin) in filtered seawater for one or a few days, rinsed in distilled water, and transferred to 70%–80% ethanol. Specimens for DNA extraction were fixed in 70% ethanol. Specimens for SEM were preserved for one hour in 1%–2% osmium tetroxide in filtered seawater, rinsed and

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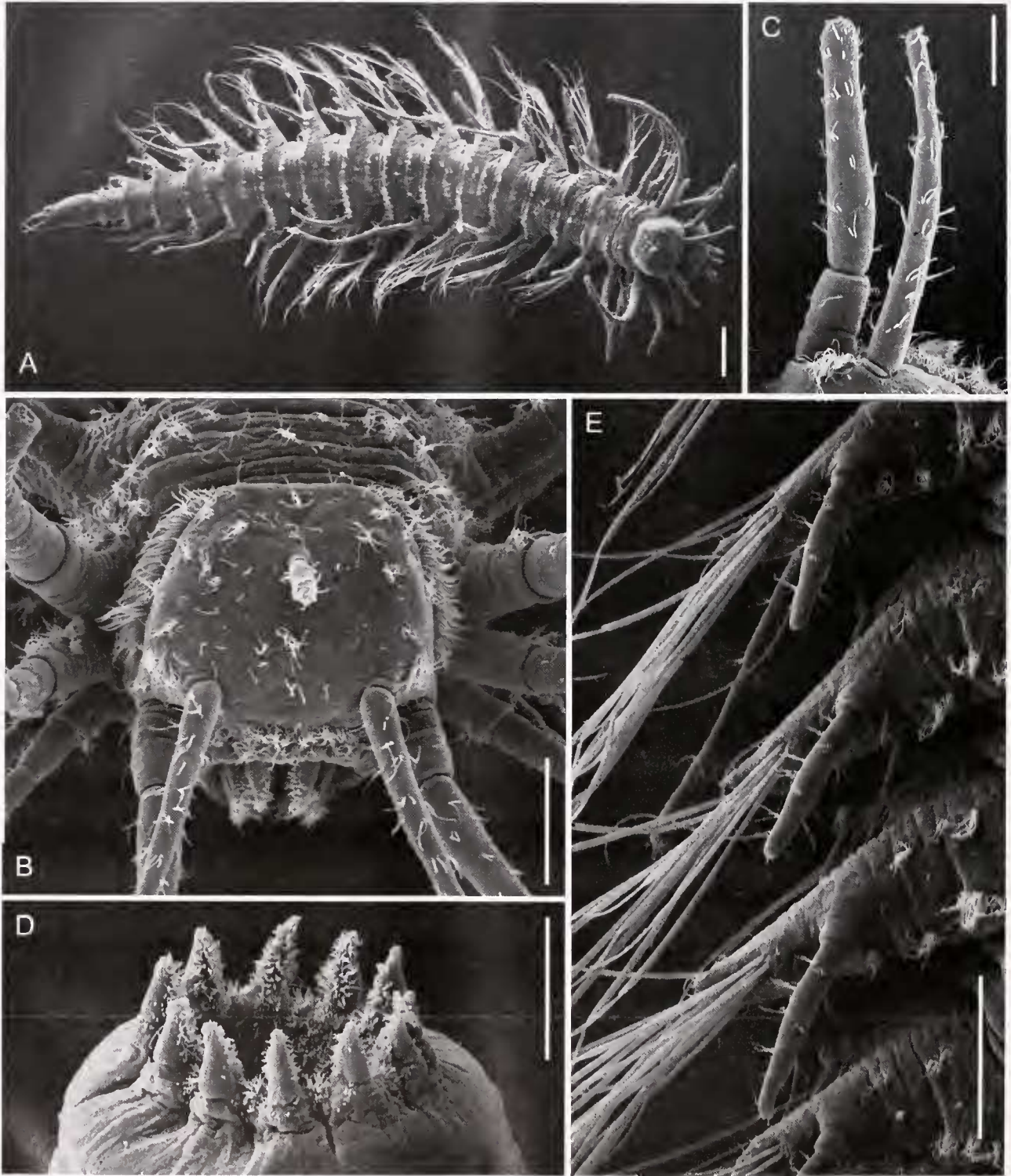


Figure 1. Scanning electron micrographs of *Lizardia hirschi*. (A) Male, dorsal view. (B) Anterior end, dorsal view. (C) Palp and paired antenna. (D) Distalmost part of everted proboscis. (E) Median parapodia, right side, ventral view. Scale lines: A, 100 μm ; B, C, 25 μm ; D, E, 50 μm .

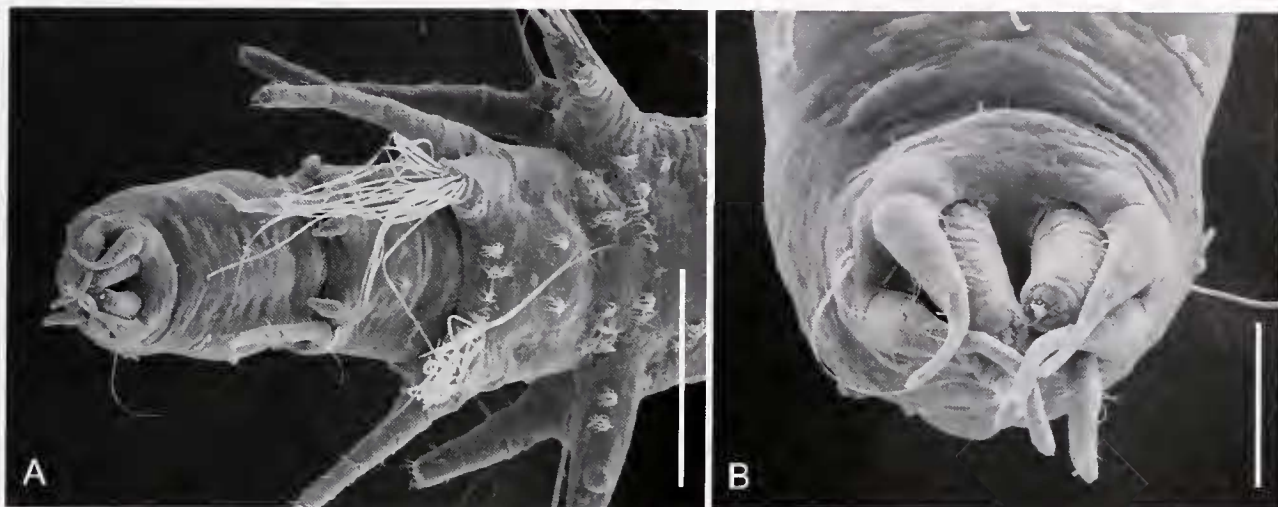


Figure 2. Scanning electron micrographs of *Lizardia hirschi*. (A) Posterior end of male, ventral view. (B) Posterior end of male, posteroventral view. The penes are the stout median projections, surrounded (from top to bottom) by ventral cirri, dorsal cirri, and pygidial cirri (partly hidden). Scale lines: A, 100 μm ; B, 25 μm .

stored in distilled water, transferred to alcohol, critical-point-dried, and sputter-coated with carbon or gold (or both); microscopy was conducted at the SEM Laboratory of the National Museum of Natural History, Washington, DC. Specimens for histology and TEM were fixed in 3% glutaraldehyde (in 0.2 M sodium cacodylate buffer), postfixed in 1% osmium tetroxide before dehydration, and embedded in Spurr's epoxy resin. Sections of 1 μm and 70–90 nm ("ultrathin") sections were made through embedded specimens with an ultramicrotome. The 1- μm sections were stained with toluidine blue solution and photographed using a Leica DMR microscope. The ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Philips CM100 transmission electron microscope. Whole mounts for LM were made from live and preserved specimens by mounting in Gurr's Aquamount, and a few additional specimens were preserved in 70% ethanol for DNA sequencing.

Formaldehyde-preserved specimens, whole specimens mounted for LM, and sections on glass slides are deposited at the South Australian Museum, Adelaide (SAM); SEM and DNA specimens are in FP's collection, and TEM sections are in GWR's collection.

Collector names are abbreviated as follows: Thomas Dahlgren (TD), Lars Jermiin (LJ), Eva Lewy (EL), Fredrik Pleijel (FP), and Greg W. Rouse (GWR).

Lizardia, new genus

Type species. *Lizardia hirschi*, new species.

Etymology. Named for the type locality, Lizard Island. Gender masculine.

Description. Monotypic; see *Lizardia hirschi*.

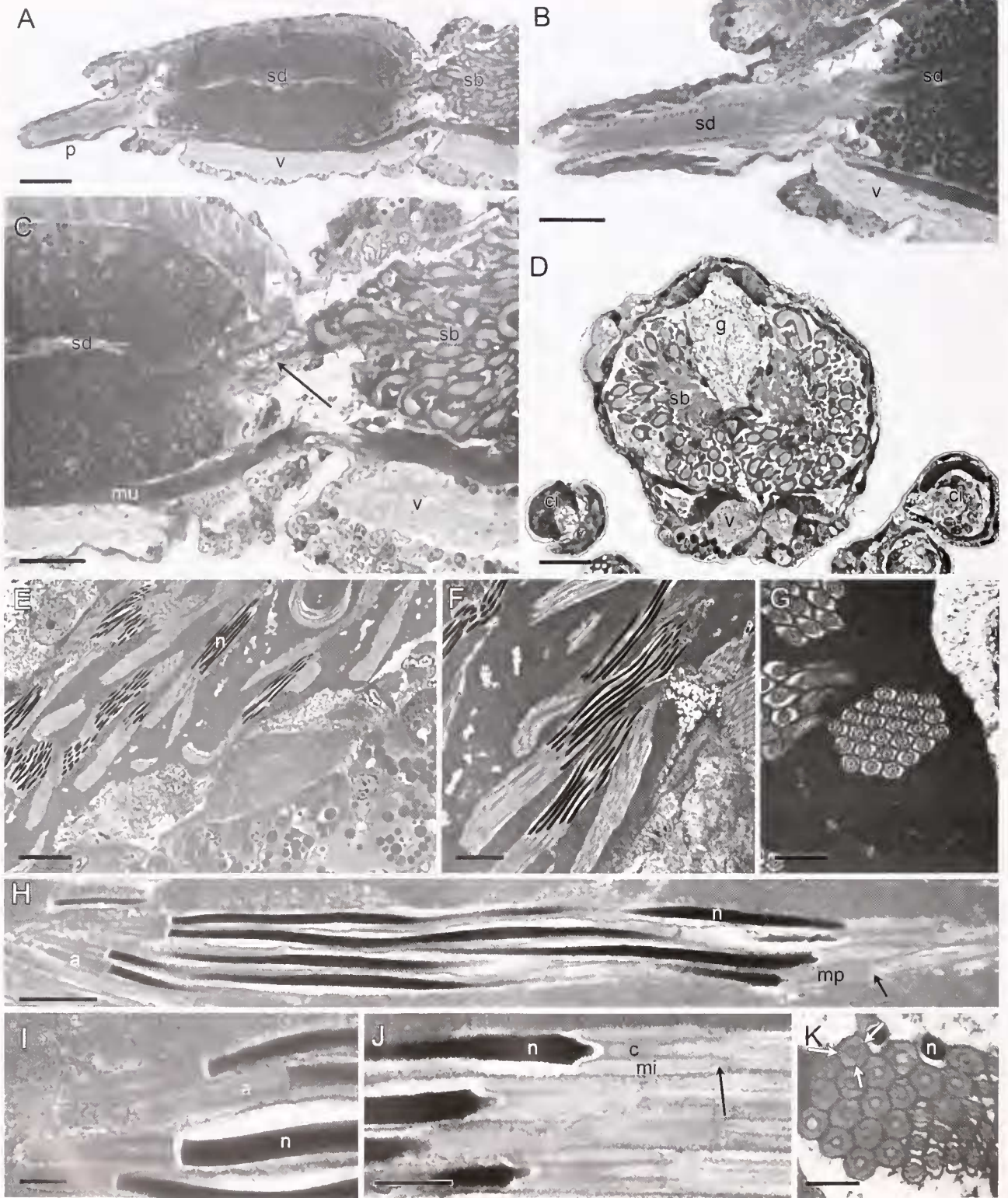
Lizardia hirschi, new species

(Figs. 1–4)

"Undescribed Hesionidae": Pleijel, 2001: fig. 18.2.

Material examined

Great Barrier Reef, Lizard Island: Holotype, immature male, fixed in formaldehyde (SAM E3366), between Palfrey and South Island, 14°42.0'S 145°26.3'E, 4 m, dead corals with algae, scuba, colls EL, FP, GWR, LJ, 27 March 2000; 1 paratype, female, fixed in formaldehyde (SAM E3363), 1 paratype mounted for LM (SAM E3364), North Point, 14°38.78'S 145°27.21'E, 8 m, coral sand, scuba, colls EL, FP, GWR, LJ, 23 March 2000; 2 spms mounted for SEM (FP), 3 spms (2 males, 1 female) preserved for LM and TEM (GWR), Bommie Bay, 14°39.13'S 145°28.031'E, 10–18 m, coral rubble, scuba, colls EL, FP, GWR, LJ, 25 March 2000; specimens for LM and TEM sectioned at 1 μm and mounted on microscope slides (SAM E3365). **Great Barrier Reef, Capricorn-Bunker Group, One Tree Island:** ca. 25 spms fixed in formaldehyde (SAM E3367), 2 spms mounted for LM (SAM E3368), ca. 15 spms mounted for SEM (FP), North Reef Flat, 23°29'S, 152°05'E, 2–3 m, scuba, colls FP, GRW, TD, among dead *Acropora*, 15 November 1996; 1 spm mounted for LM (SAM E3369), entrance Second Lagoon, 23°29'S, 152°04'E, sand and gravel, 4–6 m, scuba, colls FP, GWR, TD, 18 November 1996; 11 spms fixed in formaldehyde (SAM E3370), 2 spms fixed for DNA analyses (FP), entrance Second Lagoon,



23°29'S, 152°04'E, sand and gravel, 4–6 m, scuba, colls FP, GWR, TD, 21 November 1996; 5 spms fixed in formaldehyde (SAM E3371), "the Bommie," 23°29'S, 152°03'E, off reef, 21 m, corals and red algae, scuba, colls FP, GWR, TD, 23 November 1996; ca. 70 spms fixed in formaldehyde (SAM E3372), 6 spms mounted for LM (SAM E3373) entrance Second Lagoon, 23°29'S, 152°04'E, sand and gravel, 4–6 m, scuba, colls FP, GWR, TD, 25 November 1996; 8 spms fixed in formaldehyde (SAM E3374), 1 spm (female) preserved for LM (GWR), bay in front of laboratory, 23°30'S, 152°05'E, 1 m, among dead *Tubipora*, snorkeling, coll. FP, 27 November 1996; 1 female specimen for LM sectioned at 1 μm and mounted on microscope slides (E3375).

Etymology. Named for Dr. Len Hirsch, in recognition of his contributions to polychaete systematics.

Description

Maximum length 2.1 mm, mature males always with 20 segments (including posteriormost segment surrounding penes and pygidium), mature females with 17 segments. Body elliptical in outline with truncate anterior end and tapering posterior end (Fig. 1A). Prostomium rounded rectangular, posterior margin narrower than anterior (Fig. 1A, B), posterior incision absent (Fig. 1B). Palpophores short, cylindrical; palpostyles much longer, basally slightly inflated and tapering to rounded tips (Fig. 1C). Paired antennae as long as palps, thinner than palpostyles, evenly tapered to rounded tips (Fig. 1C). Median antenna inserted mediodorsally on prostomium (Fig. 1B), between anterior pair of eyes, much shorter than paired antenna, elliptical to tapered. Anterior pair of eyes twice as large as posterior, anterior pair round to kidney-shaped, posterior pair round, both with lenses;

anterior pair situated slightly farther apart than posterior pair, forming a trapezoid. Nuchal organs prominent, elongate, ciliated bands bordering posterodorsal and lateral sides of prostomium (Fig. 1B). Facial tubercle absent.

Proboscis smooth, jaws or teeth absent, terminal ring with 10 triangular, pointed and ciliated papillae (Fig. 1D). Proboscis extending posteriorly to segments 6–7 in non-everted condition.

Dorsal cirri and cirrophores segments 1–4 (possibly also 5) stouter and longer than on following segments, distinctly annulated. Ventral cirri segments 1–3 much stouter and longer than on following segments, distinctly annulated, situated on distinct cirrophores. Single noto- and neuroaciculae in segments 2 and 3, presence uncertain in segment 1. Segment 4 with neurochaetae, neuropodial lobes, and ventral cirri similar to following segments; notochaetae and notopodial lobes absent. Segment 5 similar to following ones.

Dorsal cirri median segments reaching about as far as notochaetae. Elevated dorsal cirri on segments 5, 8, 10, 12, 15, 17. Dorsal cirri segment 17 and thereafter directed posteriorly in males. Notopodial lobes small, conical, with double notoaciculae. Notochaetae of single kind, 5–8 fine, simple chaetae, internally camerate, with two longitudinal serrated rows with alternating teeth.

Neuropodial lobes conical, pointed, usually with single neuroacicula. Neurochaetae fine, all compound, ca. 10 each fascicle. Length of ventral and dorsalmost blades about half of median blades, with very fine and apparently unidentate tips. Shafts internally camerate. Ventral cirri inserted subdistally on small, low cirrophores, distinct annulation absent, tapering to small rounded tips (Fig. 1E), extending well beyond neuropodial lobes. No entire pair of pygidial cirri observed in females, but appear similar to dorsal cirri:

Figure 3. Reproductive system of male *Lizardia hirschi*. (A). One-micrometer longitudinal section through one of the penes, segment 20, and part of segment 19. Segment 20 contains masses of granular material and a duct for each penis. Segment 19 is filled with spermatozeugmata. (B) Detail of penis showing densely ciliated duct along its length that is continuous with the duct through segment 20. (C) One-micrometer longitudinal section through junction of segments 19 and 20. Spermatozeugmata accumulate in segment 19 and more-anterior segments and pass through to the penis via segment 20. Ciliated funnel into segment 20 indicated by arrow. (D) One-micrometer transverse section through segment 19 showing spermatozeugmata filling the coelom. (E) Transmission electron micrograph (TEM) showing spermatozeugmata in coelom of segment 19. Spermatozeugmata are surrounded by a uniform matrix of moderate electron density. (F) TEM of longitudinal section through spermatozeugmata. (G) TEM of transverse section through a spermatozeugma comprising 16 sperm. (H) TEM of longitudinal section through mature sperm, in a spermatozeugma, showing elongate cylindrical nuclei, conical acrosomes, short midpieces (terminates at arrow) and tails. (I). TEM of longitudinal section through acrosomes, which are simple, invaginated vesicles (J). TEM of longitudinal section through posterior end of sperm nuclei and midpieces. Note nuclear projection into midpiece, close to centriole apparatus. Midpiece termination indicated by arrow. (K). TEM of transverse section through a spermatozeugma showing midpieces comprising three mitochondria (arrows) and centriolar apparatus. Abbreviations: a, acrosome; c, centriolar apparatus; g, gut lumen; mp, sperm midpiece; mu, muscle; n, sperm nucleus; sd, sperm duct; sb, spermatozeugma; v, ventral nerve cord. Scale lines: A and D, 20 μm ; B and C, 10 μm ; E, 5 μm ; F, 2.5 μm ; G, J, and K, 0.5 μm ; H, 1 μm ; I, 0.25 μm .

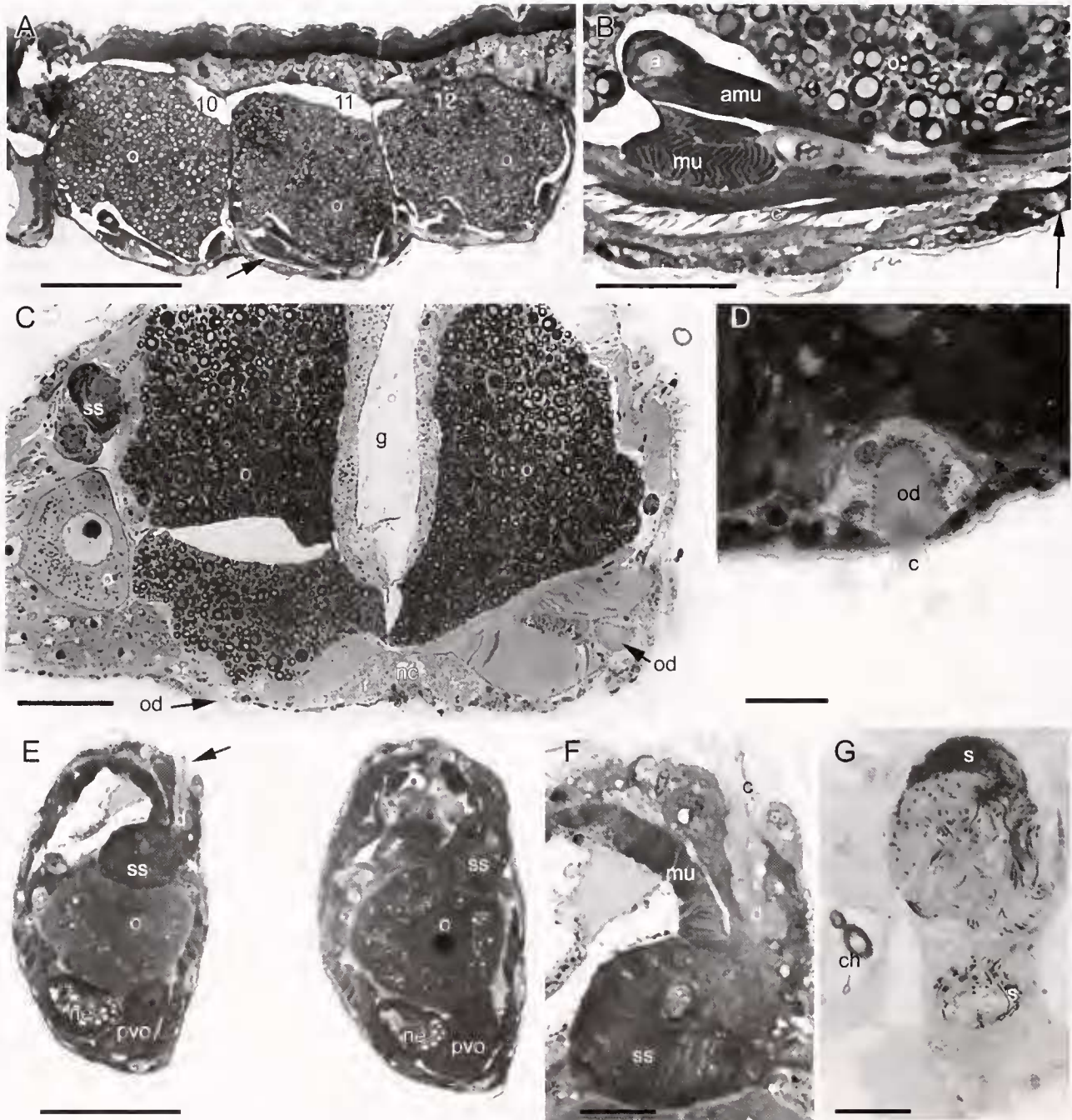


Figure 4. Reproductive system of female *Lizardia hirschi*. (A) One-micrometer longitudinal section through segments 10, 11, and 12 of a mature female from Lizard Island, showing late vitellogenic (or mature) oocytes. A pair of ciliated ducts (oviducts and/or nephridia) connect segments 10 and 11 with the funnel on the septum of segment 10 and the main duct in segment 11 (arrow). (B) Detail of sperm duct running ventrally along segment 11. Opening of the duct to the exterior (located in other sections) is indicated by arrow. (C) One-micrometer transverse section (slightly oblique) through segment 11 of female from One Tree Island, showing mature oocytes abutting gut. One spermatheca is visible, with its sperm storage area adjacent to a mature oocyte. The external opening to one of the oviducts is indicated by arrow, while the other oviduct is not emergent on this section. (D) Detail of the opening of a ciliated duct on the ventral part of segment 11. (E) One-micrometer longitudinal section through bases of parapodia in segments 11 and 12, showing a spermatheca dorsally in each. Spermathecal opening at the posterior face of the parapodium in segment 11 indicated by arrow. Oocytes or varying stages of development are visible in the coelom. (F) One-micrometer longitudinal section through spermatheca, showing duct and sperm storage area. Sperm are poorly stained in this section. (G) One-micrometer transverse section through spermatheca of female from One Tree Island, showing sperm nuclei clearly. Abbreviations: a, acicula; amu, acicula muscle; c, cilia of oviduct or spermatheca; ch, notopodial chaetae; d, spermathecal duct; g, gut; mu, muscle; nc, ventral nerve cord; ne, neuropodial chaetae; o, oocyte; od, oviduct; pvo, previtellogenic oocyte; s, sperm; ss, sperm storage area of spermatheca. Scale lines: A, 100 μ m; B, 20 μ m; C, 50 μ m; D, 10 μ m; E, 50 μ m; F and G, 10 μ m.

median pygidial papilla absent. Segment 20 in males with small dorsal and ventral cirri, with elongate tips and few, very fine and probably capillary chaetae in between; penes of about same size as cirri but truncate, inserted between ventral cirri (Fig. 2A, B) (penes may also be situated on pygidium). Pygidium in males with one pair of cirri of similar size and shape to dorsal and ventral cirri segment 20.

Pigmentation. Eyes orange. Live animals transparent, with conspicuous white pigmentation in large area across posterior part of prostomium and dorsally across segments 1–3, extending on cirrophores of dorsal cirri of these segments, as spots near bases of all parapodia, and on posteriormost region of gut. Light brown pigmentation also present dorsally as segmental stripes and blotches similar to, e.g., *Gyptis propinqua* (Pleijel, 1993). Midgut yellowish. Eggs whitish. With exception of eye pigments, preserved specimens lose all pigmentation and become opaque.

Remarks. It may be difficult to separate immature preserved specimens and females of *L. hirschi* and *capricornia*. One useful feature is the disposal of the eyes; in *L. hirschi* the four eyes form a trapezoid approaching a quadrangle, whereas in *capricornia* the four eyes are situated on a slightly curved line.

Reproductive system and internal features. Penes on segment 20 (or, possibly, on pygidium) each with ciliated ducts running through this segment (Fig. 3A–C). Penis duct lined by cilia and many granules of varying electron density. Duct of each penis continues through segment 20 before terminating as a funnel opening in posterior region of segment 19. Coelom of segment 20 otherwise completely filled with granular material of unknown composition. Funnel in coelom of segment 19 densely ciliated and extends dorsally along septal wall (Fig. 3C). Segments 16–19 contain mature sperm, packed as spermatzeugmata (Fig. 3C–G). Spermatids found from segments 9–15. Septa between segments incomplete. Mature sperm with elongate cylindrical nucleus, 8.5 μm long, capped by conical acrosome, 3.4 μm long (Fig. 3H, I). Sperm nucleus terminates posteriorly as semi-spherical projection surrounded by three mitochondria of midpiece (Fig. 3J, K). Mitochondria extend for only 1 μm behind nucleus and also surround anchoring apparatus of flagellum. Anchoring apparatus comprised of two centrioles, both abutting base of nucleus (Fig. 3J).

Females with single pair of ciliated ducts (oviducts, nephridia, or both) opening on ventral surface of segment 11, with external opening only 10 μm in diameter (Fig. 4A–D). Oocytes of various stages of development in segment 11, as well as in segments 10 and 12 (Fig. 4A, C, E), in some specimens also in segment 13. Oocytes up to 150 μm in diameter (Fig. 4A). A pair of large, late vitellogenic

or mature oocytes present in each of segments 10–12 or 10–13 in many females. Spermathecae located dorsally on posterior surfaces of each notopodia on segments 10–12 (Fig. 4C, E). Sperm embedded in spermathecal lining and tails trail along duct (Fig. 4F, G). Spermathecae appear to be blind sacs, but proximity to eggs makes internal fertilization a possibility.

Discussion

A preliminary, morphology-based phylogenetic analysis (not included here) points to a position of, first *capricornia*, and then *Lizardia hirschi*, as sister taxa to the remaining hesionids. This is in contrast to the original description of *capricornia*, where a position within Gyptini instead was obtained (Pleijel and Rouse, 2000). However, assessing the relationships of these two taxa from morphology only may be problematic due to the presence of many general hesionid characters (apart from the autapomorphies with the reproductive system). Morphologically, *capricornia* and *L. hirschi* are similar to each other, and both have a number of features that are characteristic for juvenile hesionids, including a dorsally inserted median antenna, few cephalized segments, and 10 proboscis papillae. Considering these characters, in combination with the small size of the animals, suggests the possibility that their evolution involves progenesis or neoteny and, in that case, that their indicated basal positions among hesionids may be spurious. This issue will be addressed in forthcoming studies, based also on molecular data that should be neutral *vis-à-vis* truncated ontogenies.

While the penes in *L. hirschi* and *capricornia* are clearly nonhomologous, as seen from their different positions (segment 20 or pygidium, and segment 9, respectively), the female reproductive systems of the two taxa appear to be homologous and should be treated as primary homologs in future analyses: *L. hirschi* has spermathecae dorsally on the notopodia of segments 10–12, and ventral oviduct openings on segment 11; in *capricornia* the spermathecae and oviducts are in the same position, except that spermathecae are on segments 11 and 12 only.

Among the reproductive systems of hesionids, there are other potential homologies that could be investigated. For instance, *Sirsoe methanicola* (Desbruyères and Toulmond, 1998) has a pair of sperm ducts extending through posterior chaetigers, opening into the posterior gut (Eckelbarger *et al.*, 2001). Sperm are then spawned through the anus. The possible homologous relations between the posterior sperm ducts of *Lizardia hirschi* and *Sirsoe methanicola*, notwithstanding the lack of penes in the latter group, also deserve further investigation.

Acknowledgments

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